Standard Operating Procedure for Determination of Mercury in Fish Using a Microwave Digestion or Alternate Parr Bomb Digestion Method

1.0 Location

Mercury in fish determinations are performed in the Spectroscopy Laboratory, Room 305.

2.0 Purpose

The purpose of this method is to determine the amount of mercury in fish, fillets or whole fish.

3.0 Scope

Microwave digestion or alternate Parr Bomb digestion is used to prepare fish samples for analysis by the same method used for drinking water. See SOP Method I-1-43, Determination of Mercury By Cold-Vapor Atomic Absorption for interferences, warnings, and complete analysis instructions. A closed vessel digestion, whether by microwave or Parr bomb is needed to completely digest a fish sample, since a large part of the mercury concentration consists of organo-mercury compounds including methyl mercury chloride.

4.0 Reference

4.1 AOAC Official Methods of Analysis, Fifteenth Edition, 1990, Method 974.14, pg 264.

4.2 SOP I-1-43, Determination of Mercury By Cold-Vapor Atomic Absorption.

4.3 Parr Acid Digestion Bomb Operating Instructions, No. 249M, pp 2-8.

4.4 CEM Microwave Sample Preparation System Manual for MDS-2000,CEM Corporation, 1991.

5.0 Sample Handling and Preservation

Fish samples are received ground and well mixed in glass jars. The samples are stored frozen and thawed for use. No preservatives are used.

6.0 Apparatus and Materials

6.1 Microwave digestion

6.1.1 CEM Microwave-MDS-2000

6.1.2 Lined digestion vessels: Teflon inserts and tops, acid washed and rinsed with deionized water. Outer containers and tops, rinsed with distilled water and completely dry before using. Rupture membranes, vent stems, vent tubes, pressure cap with ferrule nuts and plugs.

6.1.3 Analytical balance

6.1.4 Water dispenser capable of dispensing 10-50 ml.

6.2 Parr Bomb digestion

6.2.1 Parr bomb apparatus with inner teflon container. Acid rinse teflon container before each use. See section 9.13.

6.2.2 Spanner and holder

6.2.3 Muffle furnace

6.2.4 Plastic bottles and caps

6.2.5 Analytical balance

6.2.6 Water dispenser capable of dispensing 10 ml and 20 ml

6.2.7 Ice bath

6.3 General apparatus and materials

6.3.1 BOD bottles, glass with stoppers and plastic caps

6.3.2 Perkin-Elmer 3100 Atomic Absorption Spectrometer

6.3.3 Mercury Electrodeless Discharge Lamp (EDL).

6.3.4 Electrodeless Discharge Lamp (EDL) power supply.

6.3.5 Absorption cell.

6.3.6 Cell support.

6.3.7 Air pump (capable of delivering 1 liter of air per minute) and in-line regulator.

6.3.8 Glass aerator.

6.3.9 Connecting tubing (interconnects all components).

6.3.10 Drying tube (Desiccant).

6.3.11 Analytical balance.

6.3.12 Volumetric flasks.

6.3.13 Graduated cylinders.

6.3.14 Eppendorf pipet.

6.3.15 Waterbath and thermometer.

6.3.16 NOTE: All glassware should be acid soaked with a 30% nitric acid solution over night; then triple rinsed with distilled water and twice with ASTM Type II water or better.

7.0 Reagents (All reagents must be suitable for mercury analysis)

7.1 Deionized water.

7.2 Sulfuric Acid (concentrated, H_2SO_4), suitable for trace metal analysis.

7.3 Nitric Acid (concentrated, HNO_3), suitable for trace metal analysis.

7.4 5% Potassium permanganate solution (W/V): Dissolve 50g of potassium permanganate in water and dilute to 1 L.

7.5 5% Potassium persulfate solution (W/V): Dissolve 50 g of $K_2S_2O_8$ in water and dilute to 1 L.

7.6 10% Hydroxylamine hydrochloride solution (W/V): Dissolve 100 g of hydroxylamine hydrochloride in DI water and dilute to 1 L or use solid

hydroxylamine hydrochloride adding approximately 0.5g with measuring spoon to each bottle.

7.7 10% Stannous chloride solution (W/V): Dissolve 50 g of $SnCl_2$ in 50 mL of concentrated hydrochloric acid (HCl), with heating if necessary until clear, dilute to 500 ml. On aging, this solution decomposes. If a suspension forms, mix reagent before use and continuously while using.

7.8 Magnesium perchlorate (used as a desiccant in the drying tube).

7.9 Stock Mercury Atomic Absorption Standard Solution, purchased from a reputable commercial source such as SPEX in a concentration of 1000 ug/ml.

7.10 Working Mercury Standard, 1 ppm (1ug/ml) Add 100 ul of the 1000 ppm Stock Mercury Standard Solution to a 100 mL volumetric flask containing 90 mL of deionized water and 2 ml HNO_3. Dilute to volume with deionized water and mix well. Prepare fresh for each use. Use aliquots of this solution to prepare standards, see section 8.3.2.

7.11 Working mercury spike - prepare as in 7.10 using a different Stock Mercury Standard. The usual concentration for spiking is 300 ug/L Hg added achieved by adding 300 ul of working spike solution.

7.12 12 % nitric acid solution.

7.13 Marine Reference Material: Dorm-1, dogfish muscle, from the National Research Council of Canada

8.0 Procedures

8.1 Microwave Digestion Procedure

8.1.1 NOTE: Before beginning procedure read CEM Microwave Sample Preparation System Manual - General safety considerations pp. I3-I5; Part III Start Up; Part IV Operation; Part V Maintenance; and Appendix on lined digestion vessels.

8.1.2 Weigh a 0.5000 g sub-sample into a teflon insert. Record the weight to the nearest 0.1 mg. on printed spreadsheet. Do each sample in duplicate. One vessel, the pressure vessel, should contain a larger sample approximately

0.6000 g.

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8.1.3 Spike at least 10% of the samples.

8.1.3.1 Matrix spike: Spike by adding 0.3 ug (300 ul) of spiking solution to the sample in the inner teflon container before digesting. The amount spiked should be near the expected concentration of mercury in the sample when possible.

8.1.3.2 Calculate the amount added by dividing the amount in micrograms, 0.3 ug added, by the weight of the sample, in grams, to obtain ug/g added.

8.1.4 Do one blank and one LFB (lab fortified blank) spiked at the same level used for the matrix spike for each set of 10 samples.

8.1.5 Place the insert into the outer container. Record outer container number. Add 9 ml of conc. nitric acid to each vessel. Repeat for each sample. 12 sample containers work best for this method.

8.1.6 Cap and assemble complete digestion vessel according to microwave reference directions.

8.1.7 Place, evenly spaced, on turn table and put in microwave.

8.1.8 Fill syringe with de-oxygenated water and rinse pressure line with about 40 ml water into waste container. Pressure valve should be in open position to rinse.

8.1.9 Attach pressure line to most reactive sample (heaviest weight) with special pressure cap with new rupture membrane. The pressure line should go through the center post and should not interfere with circulating fan during table rotation. Make sure line is completely filled or pressure sensor will not work. If pressure line does not seem to hold pressure, an O-ring can be inserted in the ferrule for the pressure line. For monitoring the temperature use the cone shaped ferrule nut only for the armored temperature probe. Insert glass thermowell and turn on cone shaped ferrule and then insert probe, making sure it is inserted completely and held firmly by ferrule. Make sure lines are free and do not interfere with the rotation of the turntable. Both pressure and temperature should be monitored in order to prevent high temperature damage (pinholes and over

expanding) to vessels. When not using the temperature probe, close off temperature control port with a sealed ferrule nut.

8.1.10 Place regular teflon lids with new rupture membranes on remaining vessels.

8.1.11 Check that the pressure line does not interfere with the rotation of the turn table.

8.1.12 Turn pressure valve to neutral or closed position for microwaving.

8.1.13 In Main Menu select Recall Stored Method and select Fish Tissue. Check that the method looks like the following:

Stage	1	2	3	4	5
Power	100	100	100	100	
PSI	50	90	130	170	
Time	20:00	20:00	20:00	10:00	
TAP	5:00	5:00	5:00	8:00	
Temp	150°C	180°C	180°C	190°C	
Fan	100%	100%	100%	100%	

Additional information which is included in the method but does not affect the operating parameters:

Vessels 12 Sample Weight 0.5 g
Volume/vessel 9 ml Acid HNO_3

8.1.14 Change power level when changing the number of vessels used according to the table below:

No. of vessels	Power level
2	40%
3	45%
4	50-55%
6	60%
8-12	100%

8.1.15 Make final check including: new rupture membranes have been installed, all caps, vent stems, and ferrules are tightened, pressure control line is connected, pressure control valve is in closed position, vent tubes are in, turntable turns without obstruction, temperature probe is inserted properly and line is out of the way. With the door open press F4 to start the turntable rotating to check that all lines are out of the way and turntable turns freely. Make sure door closes properly.

8.1.16 Begin digestion by pressing F4 to start. Check that pressure rises and turntable rotates freely. Press F2 to print run time data, this should be saved along with other data.

8.1.17 When method is completed, open and close door to stop rotation of turntable. Allow pressure to drop to between 30-60 psi before venting pressure sample and removing pressure line or use special pressure valve which allows immediate removal. Move entire turntable to hood and allow to cool with ventilation.

8.1.18 Carefully, vent each vessel. Transfer contents carefully to BOD bottles with 3-30 ml rinses. Rinse cap and inner microwave vessel. Stopper and cap BOD bottle. Store in refrigerator, approx. 5°C until ready to analyze. Continue with section 8.3 Analysis Procedure.

8.2 Alternate Parr Bomb Digestion Procedure

Caution: Do not increase amount of sample or acid as an explosion is possible when heating in the bomb apparatus.

8.2.1 Preheat muffle oven to a constant 150° C± 5.

8.2.2 Weigh 0.5000 grams of sample, after thawing, directly into prepared inner teflon container.

8.2.3 Add 2.5 ml of concentrated nitric acid directly to sample in teflon container, cover.

8.2.4 Assemble inner container and bomb apparatus.

8.2.5 Tighten with spanner and holder.

8.2.6 Place in oven. Record time and temperature.

8.2.7 Remove after one hour. Record time and temperature.

8.2.8 Cool under hood for half hour.

8.2.9 Place in pan in ice bath to complete cooling, about 20 minutes.

8.2.10 Under hood, open bomb, remove teflon container.

8.2.11 Transfer digested sample to a plastic bottle with four 10 ml rinses of deionized water. Be sure to rinse cover also.

8.2.11.1 Label with log number, weight, and date of digestion.

8.2.11.2 Store plastic bottles in refrigerator at approximately 4° C until ready to analyze.

8.2.12 Transfer sample from plastic bottle to BOD bottle with three 20 ml rinses of deionized water.

8.2.13 Wash teflon container with soap, rinse, acid rinse with nitric solution, rinse with distilled water, rinse with deionized water and dry before each use.

8.2.14 Repeat from 8.2.1 to 8.2.12 for each fish sample in duplicate and for each check sample in duplicate.

8.2.15 Spike at least 10% of the samples.

8.2.15.1 Spike by adding 0.3 ug (300 ul) of spiking solution to the sample in the inner teflon container before digesting. The amount spiked should be near the expected concentration of mercury in the sample.

8.2.15.2 Calculate the amount added by dividing the amount in micrograms, 0.3 ug added, by the weight of the sample, in grams, to obtain ug/g added.

8.2.16 Do one blank at beginning of digestion phase.

8.2.17 Continue with section 8.3. Analysis Procedure.

8.3 Analysis procedure

8.3.1 Remove samples in BOD bottles from refrigerator.

8.3.2 Prepare these standards from the working mercury standard solution as prepared in 7.10. To 100 ml deionized water in a BOD bottle add:

Aliquot Added (in ul)	Concentration (ug)
0	0.00
50	0.05
100	0.10
300	0.30
500	0.50
800	0.80
1000	1.00

8.3.3 Continue with the analysis instructions section 10 in SOP Method I-1-43, Determination of Mercury By Cold-Vapor Atomic Absorption.

9.0 Quality Control

9.1 Samples are always analyzed in duplicate and the results averaged.

9.2 Spike at least 10% of the samples with a minimum of one spike per run.

9.2.1 Matrix spike: Spike by adding 0.3 ug (300 ul) of spiking solution to the

sample in the inner teflon container before digesting. The amount spiked should be near the expected concentration of mercury in the sample if possible.

9.2.2 Calculate the amount added by dividing the amount in micrograms, 0.3 ug added, by the weight of the sample, in grams, to obtain ug/g added. Recovery of the spike must be within 70-130% or samples must be rerun.

9.3 One marine reference sample, Dorm-1, is analyzed with each 10 samples. Recovered mercury concentration of this sample must be within acceptable limits or samples must be rerun.

9.4 One blank and LFB (lab fortified blank) spiked at the same level as the matrix spike are analyzed with each set of samples.

10.0 Data Analysis

10.1 Construct a curve by plotting the absorbance readings of the standards against the concentration of the standards in micrograms (ug) of mercury.

10.2 Compare each sample's maximum absorbance value to the standard curve to obtain the concentration of mercury in each sample in micrograms.

10.3 Calculate the mercury concentration for each sample in ug/g:

ug Hg/g = ug Hg in sample/ g of sample used

10.4 As an alternative to the above calculation, a calculator employing linear regression may be used to determine the mercury concentration of the samples.

10.5 For this method an Excel spreadsheet is used for all calculations. The correlation of coefficient should be 0.99 or better or samples must be rerun.

10.6 If the concentration is less than 0.1 report as < 0.1 ug/g, if less than or equal to 1 report to the nearest hundredth, if greater than 1 or equal to 10 report to the nearest tenth, and if greater than 10 report to the nearest ug/g.

11.0 Documentation

11.1 All sample information, weights, and values are recorded on a printed Excel spreadsheet and saved. Run time data printouts are saved.

11.2 Parameter information is saved.

11.3 Print outs of absorbance data for standards and samples are saved.

11.4 Computer calculation print outs are saved.

11.5 Microwave digestion temperature and pressure printouts are saved.

11.6 LMS printouts are saved.

12.0 Records

Printed Excel spreadsheets, parameter information, run time data, microwave digestion data, absorbance data, LMS printouts, and computer calculations are stored in the Mercury in Fish 3480 logbook in the bookcase in Room 305. Records are transferred to boxes for long term storage in a proper facility.